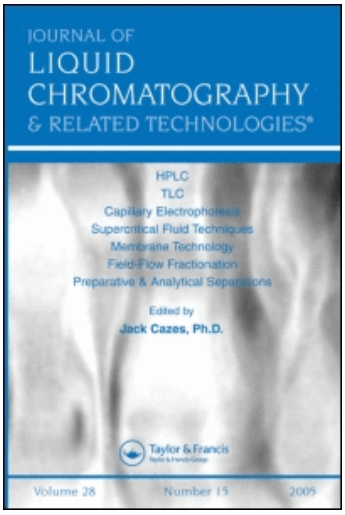

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Molecular Recognition of Acyclic Stereoisomer Using a Simple High Performance Liquid Chromatography. I

Ken Hosoya[a]; Kazuhiro Ktmata[a]; Takeo Araki[a]; Nobuo Tanaka[a]; Kunihiko Takabe[b]; Hidemi Yoda[b]
[a] Department of Polymer Science, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto, Japan
[b] Department of Engineering, Sizuoka University, Johoku, Hamamatsu, Japan

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MOLECULAR RECOGNITION OF ACYCLIC STEREOISOMER USING A SIMPLE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. I.

KEN HOSOYA[1]*, KAZUHIRO KIMATA[1], TAKEO ARAKI[1],
NOBUO TANAKA[1], KUNIHIKO TAKABE[2],
AND HIDEMI YODA[2]
[1]*Department of Polymer Science*
Kyoto Institute of Technology
Matsugasaki, Sakyo-ku, Kyoto 606, Japan
[2]*Department of Engineering*
Sizuoka University
Johoku, Hamamatsu 432, Japan

ABSTRACT

Separation of stereoisomer such as diastereomer on silica gels was carried out using high performance liquid chromatography (HPLC) in a normal phase mode to investigate a correlation between the configuration of diastereomers and their elution order. Relatively flexible acyclic alcohols including a variety of functional groups such as amide, nitro, ester, sulfone, or sulfoxide are employed as solutes diastereomers because of their synthetic importance and analytical interest. The diastereomers can be easily resolved on various types of silica gels under a typical normal phase condition but unexpectedly, their elution order tends to be classified into two different types mainly based on a substituent group. Silica particles containing various metals were also utilized to increase the interaction between adsorbents and solutes through a chelating effect and the metals seem to affect not only elution time but also resolution of diastereomer based on the type of a substituent group. Through experiments to change the conformation of diastereomer through preventing intramolecular hydrogen bonding by a bulky protective

group for the hydroxy group, the elution order of diastereomer is found out to be affected mainly by both sterical bulkiness of diastereomers derived from their stabilized conformation and the retention selectivity of the substituent group therein in the employed mobile phase. Finally a certain prediction for the determination of configurational information of acyclic alcohol diastereomers can be made from their chromatographic information. The results may be useful in developing further the theory and/or rules of selectivity in separation of stereoisomer using a simple chromatographic method.

INTRODUCTION

Determination of the structures of stereoisomer has been one of the most important and interesting (but time-consuming) procedures in both organic and analytical chemistry. Especially, for organic synthesist, it is not avoidable in synthetic pathway to get a desirable final product. Enormous progresses have been made in analytical equipments and techniques but in general, neculear magnetic resonance (NMR) is thought to be one of the most applicable and reliable methods to obtain both configurational and conformational informations of organic compounds. In NMR analysis, a configurational information can be obtained according to the difference in a structural interaction between substituent groups which simply results in some differences in their chemical shifts or coupling constants. In some cases, the ratio of produced diastereomers as well as the purity of the products can be also determined from NMR information such as peak integration according to the difference in chemical shifts of a substituent group of isomers, however, small change in the chemical sifts results in a complicated and sometimes only combined one broad peak for a mixture of the diastereomer. In an advanced synthetic chemistry, although the isomer ratio is very important for the purity of products, a quite high isomer ratio higher than 99 : 1 is often reported only according to NMR data. As described later, it seems to be very liable for misunderstandings of real purity of stereoisomer with a quite high isomer ratio.

High performance liquid chromatography (HPLC) must be able to detect a very small impurity and for this purpose, the sensitivity of detectors has been trying to be improved day by day especially in bioscientific separation field. This sensitive analytical technique is thought to be suitable only for a quantitative analysis because unfortunately only one peak is obtained from one compound with very high theoretical plate number. However, in fact, most of the persons connected with HPLC empirically know a possible elution order of a series of sterically similar compounds and developmentally try to expect a certain configuration of a serious of the stereoisomers based on their elution order and sometimes, this kind of prediction often makes their total experiments easier and faster.

Recently, a few attempts were made for a prediction of the structures of regioisomeric and/or diastereomeric compounds using this sensitive method for quantitative analysis (HPLC) [1] and a satisfactory correlation between the structures and the elution order seems to be obtained mainly for cyclic compounds [2]. Since in chromatographic separation, a dynamic interaction between stationary phase and solute is a dominant separation mechanism, it is much easier to achieve such a work with cyclic isomers because a conformational rigidity tends to be guaranteed for cyclic compound. Actually, substituted cyclohexanes were resolved on specially designed stationary phases in our laboratory and some possible prediction has been proposed [3]. Therefore HPLC seems to be very useful also for molecular recognitions of stereoisomers.

In addition, most of synthetic organic chemists traditionally use chromatographic techniques for purification of products, in other words, separation of a desired compound mainly in a normal phase mode with simple silica gels or aluminas and their thin layer chromatography. Sometimes, highly complicated compounds such as diastereomers can be separated each other using a simple open column chromatography and a certain prediction of configurational information is tried to be obtained from their elution order because for the same type of compounds it is not always incorrect that the change of aliphatic side chain can hardly affect the elution order on the same adsorbent in

the same mobile phase [4]. However, their chromatographic knowledge is of course limited to that of the compounds in daily use.

Nowadays, stereoselective or stereospecific reactions are required for the syntheses of naturally occurring compounds and the scientific interest have turned to stereo controls of acyclic compounds from that of cyclic compounds because of their difficulties in control. The desirable pure enantiomer or diastereomer can be occasionally obtained by some highly controlled chemical process without any separation process [5], however, in most cases, a certain separation process and the quantitative analysis of the processed compounds are essential to obtain really stereochemically pure compounds because the absolute purity in the stereochemistry must be required for syntheses of most of chiral drugs [6]. Actually, most of synthetic organic chemists have separated diastereomeric isomers by simple column chromatography with a marvelous technique, because diastereomer is only stereoisomer which can be separated on simple silica gels with small money except the stereoisomers of substituted olefines. Since synthetically interesting compounds is taking up various topics, a lot of chromatographic information are required for organic chemists, in other words, some analytical chemists must deal with synthetically important compounds not biopolymers and make attempts to get not only quantitative information but structural information using HPLC. This might be a good go-between and one of another directions of modern HPLC. For example, recently, the separation of a series of Buckminsterfullerenes can be made by chromatographic method and this separation step plays an important role for the further research of them [7].

Since acyclic compounds have interested a majority of organic synthetists as described earlier, we try to make a resolution of such a compound by a simple HPLC and some attempts to determine and recognize the structures of acyclic stereoisomer using a simple HPLC technique because the easily acceptable and cheap technique is really required for organic synthetists. Although some good HPLC columns are now available, those are clearly more expensive than silica gel columns.

From this point of view, β-substituted acyclic alcohols were chosen as the stereoisomer applied in this primary work. β-substituted acyclic alcohols potentially include various substituents, therefore, this type of compounds have been utilizing as an intermediate for the syntheses of lots of naturally occurring compounds [8]. In addition, recently, enzymatic reductions of α-substituted carbonyl compounds are being watched with keen interest [9] and the measurement of diastereomeric ratio is also a very important factor to estimate the reaction pathway. Therefore it is also a good target for analytical chemists Usually, the determinations of such diastereomeric isomers have been done using gas-liquid chromatographic method, however, thermal isomerization of compounds can not be negligible and preparative scale separation can hardly take place through gas-liquid chromatography.

Again, in this report we wish to report separations of β-substituted acyclic alcohols with a variety of substituent groups in a normal phase mode and the relationship between their configuration and their elution order is discussed. In normal phase mode, hydrogen bonding that is very important for molecular recognition works better than that in a reversed phase mode and is one of the most significant interactions between solute and stationary phase. In addition, intramolecular hydrogen bonding can fix the conformation of diastereomers somehow that may be a good assistance and very suitable for a molecular recognition of acyclic stereoisomer.

EXPERIMENTAL

Materials : All β-substituted acyclic alcohols were prepared in our laboratory using a typical preparation method described elsewhere [10]. The configuration of diastereomers was determined by NMR technique according to the reported method [11] after an isolation procedure of each diastereomer.

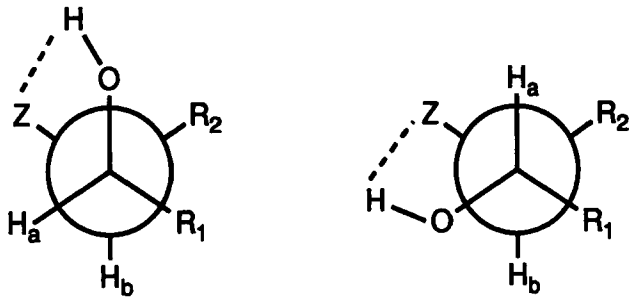
All chromatographic grade solvents were purchased from Nacalai Tesque (Kyoto, Japan) and used without further purifications. Hypersil™ and Kromasil™ were

purchased from Shandon and Eka Nobel, respectively, while Develosil™ was purchased from Nomura Chemical. Another processed silica gels were gifts from Nacalai Tesque where they were prepared and a typical acid washing took place before use. An ICP analysis was carried out at Nacalai Tesque. All silica particles were packed into stainless-steel columns (4.6 mm I.D. X 150 mm) under a wet condition.

Equipments : Chromatography was carried out with a Jasco 880-PU intelligent HPLC pump equipped with a Rheodyne 7125 Valve loop injector. Both UV detector of a Jasco UVIDEC-100-III and RI detector of a Waters Differential Refractometer R401 were used for peak monitor. Columns were thermostated at 30 ± 0.1 °C and all measurements using the same mobile phase were finished within the working time of a day because the conditions of mobile phase such as water content or exact mixing rate of binary solvent system must sensitively affect the elusion of samples in a normal phase mode. t-Butylbenzene was utilized for the t_0 measurement and capacity factor k' was calculated based on the retention time determined using a Shimadzu C-R4A chromatopac. Reproducibility of data in duplicate was better than 1 %.

RESULTS AND DISCUSSIONS

β-substituted acyclic alcohols were reported to possess intramolecular hydrogen bonding between the hydroxy group and a substituent at β position of the hydroxy group, when this substituent at β position includes hydrogen acceptor [12]. This effect namely "*gauche effect*" tends to prevent a conformational free rotation to give a relatively fixed conformation overcoming even steric repulsion between both alkyl substituents (Figure 1) [11]. This stabilized gauche conformation is confirmed by the differences in ^{1}H-NMR coupling constants of protons between H_a and H_b as shown in Figure 1 where threo isomer usually showed larger coupling constant than erythro isomer. In addition, chemical shift of the same proton in ppm was slightly different between threo isomer and



erythro *threo*

FIGURE 1. *Gauche* conformation of β substituted alcohol

erythro isomer. Utilizing this difference, diastereomer ratio is able to be calculated theoretically, however, in fact, peak broadening found in the diastereomer mixture prohibit acceptable determination of isomer ratio, especially in the case of a shifted isomer ratio.

Figure 2 demonstrates 200 MHz ^{1}H-NMR spectrum of 3-hydroxy-2-phenylsulfonyl undecane, where the dominant diastereomer is threo form in 94 % d.e.. The peak at 3.18 ppm (q,d,) demonstrated coupling constant J = 7.33 Hz and 7.02 Hz which clearly indicated this diastereomer was the threo form [11]. On the other hand, no peak derived from the minor isomer erythro form could be hardly found out on the normal size NMR spectrum, while the enlarged figure only showed very small peak like a broad noise around 3.03 ppm that was derived from erythro form. Obviously, it is dangerous to determine a diastereomeric excess from this NMR spectrum. On the contrary, a HPLC analysis can easily determine such a high diastereomeric ratio if the diastereomer is well resolved. Therefore if some configurational information can be obtained from the elution order in HPLC separation it may become one of the most important method for both synthetic organic chemists and analytical chemists because in this case, NMR analysis

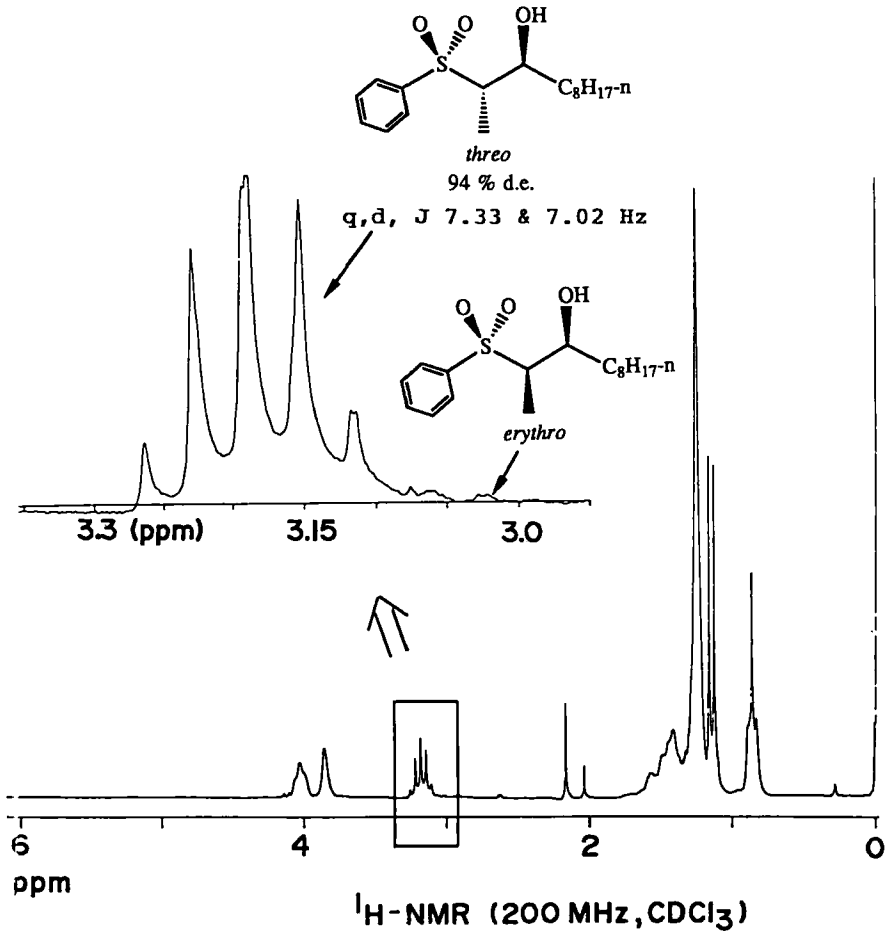


FIGURE 2. ^{1}H-NMR spectrum of 3-hydroxy-2-phenylsulfonyl undecane

can no longer give diastereomeric excess. Good advantages of HPLC method may be high sensitivity which can make a determination of isomer ratio easier and an actual separation of stereoisomer which is not avoidable in synthetic process. In order to attain the faint purpose, massive data should be required. However, first of all, effects of the substituent on the elution order of acyclic diastereomer is investigated because the substituents may make a dominant difference in the retention time in a normal phase mode.

From the viewpoint of synthetic chemistry, an electronwithdrawing group is preferred as a substituent group at β position of hydroxy group because of possibilities for the further constructions of carbon-carbon bonding. In this paper, we utilize typical important electronwithdrawing groups such as amide, sulfone, nitro, sulfoxide, and ester groups. These functional groups include oxygen atom as a hydrogen acceptor and form six member type coordination to afford gauche conformation as described earlier [12]. In a normal phase mode, these groups may act as relatively hydrophilic group which delay the elution time. Since the difference in interactions between the solutes and the stationary phase create the separation of stereoisomer, it is very important to determine retention behavior of each functional group in a normal phase mode. Table 1 showed retention behavior of the substituted ethane on silica columns. These values are relative capacity factor α calculated based on capacity factor of phenylsulfonyl ethane.

According to a reported elusion order [13], a compound including amide group was retained longer on silica gels than that including sulfoxide group, however in the employed mobile phases, phenylsulfinyl ethane showed the largest α value in all substituted ethanes which suggests an interaction between sulfoxide group and adsorbent (may be silanol) is the strongest within these compounds. That is normal because electron rich oxygen atom and a electron lone pair may tightly interact with active site on adsorbent, in this case, silanol which is an acidic functional group. Moreover, usually, silica gels including metal ions may demonstrate a certain acidic characteristics depending on the metal characteristics and interact with basic compound or hydrogen donor more

TABLE 1. Retention Behavior of Functional Groups.

Z	α ($k'_Z/k'_{sulphonyl}$)		
	Hypersil™	Develosil™a)	Develosil™b)
NO_2	0.24	0.33	0.26
$PhSO_2$	1	1	1
$PhSO_2$c)	1	1	1
PhSO	6.48	5.40	6.64
PhSOc)	3.36	2.81	3.47
PhNHCO	1.20	1.31	1.32
PhNHCOc)	2.62	2.23	1.99
CH_3OOC	0.09	0.14	0.09
PhOOC	0.09	0.21	0.18
CH_3O	0.04	0.05	0.16
$PhCH_2$	0.01	0.01	0.01
PhS	0.03	0.05	0.04

a) acid washed. b) not acid washed. c) mobile phase = iso-propanol : hexane = 5 : 95 (v/v), another cases ; Ethyl acetate : hexane = 20 : 80 (v/v).

tightly. Therefore, phenylsulfinyl ethane retained longer on silica gels without the acid washing which includes a lot of metals as impurities as demonstrated later (Table 2) because of the preparation procedure of silica gel. Interestingly, although amide group seems to be very hydrophilic functional group, it showed a similar retention behavior to corresponding phenylsulfonyl group and the retention was slightly longer on acid washed silica gels. That is mainly due to the weakly proton donor characteristics of the amide group because phenylsulfinyl ethane showed shorter retention time on the acid washed silica gel, while there were almost no differences in the functionalized ethanes not including an oxygen atom.

When iso-propanol was employed as a strong solvent in the mobile phase, phenylsulfinyl ethane eluted relatively faster than that in ethyl acetate as a co-mobile phase, while propionic anilide (amide) showed relatively longer retention time in iso-propanol than that in ethyl acetate. This drastic change may be due to the property of amide group as a hydrogen donor. According to a retention mechanism in normal phase mode [14], hydrogen bonding between solutes and adsorbents is the most effective driving force and also competitive replacement between mobile phase molecules and

solutes on the adsorbents is important. In ethyl acetate, mobile phase molecules may interact with adsorbent more tightly than the amide group because ethyl acetate is a hydrogen acceptor, while iso-propanol can hardly interact with pure adsorbent because of its hydrogen donor property. These explains suggest in the case of amide group, propionic anilide less interacts with adsorbent in ethyl acetate than dose the solvent molecule, while in iso-propanol, the solute can interact with adsorbent more tightly, because α value in the mixture of iso-propanol and hexane is larger than that in ethyl acetate-hexane. On the other hand, in the case of phenylsulfinyl ethane, expected strong interaction between iso-propanol and sulfoxide group in other words, solvation with iso-propanol may make the hydrophilicity of the solute smaller. However, overall, kinds of silica supports did not affect significantly on retention behavior. In chiral separation, an amide group seems to be the most effective group to form hydrogen bonding between a solute and a stationary phase that is mainly due to both hydrogen donor and hydrogen acceptor characteristics of an amide group [15], however, from the viewpoint of hydrophilicity which means strong interaction, phenylsulfinyl group seems to be more hydrophilic than corresponding amide group, which can potentially promote a good resolution.

For the actual separation, 3-hydroxy-2-substituted undecanes were employed as solutes (Figure 3). These compounds include relatively long saturated alkyl chain which is bulky enough but not necessarily suitable group for a molecular recognition in a normal phase mode, because aliphatic group can hardly make significant change on the sample retention, however, these compounds are very useful intermediates in synthesis of various naturally occurring compounds and their diastereomeric ratios have an important responsibility for the further syntheses and the final targets. If the discuss is limited to one substituent group, for example, diastereomeric 3-hydroxy-2-phenylsulphonyl alkane, the erythro isomer is eluted faster than the threo isomer on silica gels in every case [10]. Therefore, if the elusion order is changed each other in this

threo *erythro*

FIGURE 3. Configuration of 3-hydroxy-2-substituted undecane

series of alcohol derivatives, that is mainly due to the effect of substituent group at β position of the hydroxy group as demonstrated in Table 1.

Silica gels potentially include various metal impurities because of their preparation process and that is the reason why an acid washing is essential for the good reproducibility. Here, we utilized various types of silica gels to investigate their ability for molecular recognitions. Table 2 showed the silica gels utilized in this report and their physical properties. Unexpectedly, Hypersil included relatively high metal impurities as well as Develosil without acid washing. These high metal impurities in Hypersil may explain the similar retention tendency of substituted ethanes to the silica gels without acid washing (Table 1).

Usually, β-keto carbonyl compounds such as acetyl acetone are found to interact with metal ions through a chelate formation to delay elution and peak broadening even in a chromatographic mode. If this works for the β-substituted alcohols, Lewis acids such as $AlCl_3$ or $TiCl_4$ may fix the conformation of diastereomers to improve the molecular recognition. Although the state of the metal in silica gels is not so clear, that is the aim to introduce metal ions into silica gels. Table 3 showed the results of the separation of diastereomers on silica gels.

TABLE 2. Physical Properties of the Used Silica Gels

Name	Particle size (μm)	Mean Pore size (nm)	Metal Contents (ppm)[a] Al	Ca	Fe	Ti	Zr
Hypersil	5	10	202	100	153	39	90
Kromasil	5	10	0	0	0	2	0
Develosil[b]	10	10	107	748	25	119	18
Develosil[c]	5	10	54	215	0	38	5
Sil(Al)[d]	5	10	373	325	0	49	9
Sil(Ti)[e]	5	10	36	250	0	201	8

a) Determined based on an ICP analysis. b) Not acid washed. c) Acid washed. d) The silica gels were treated with $AlCl_3$, then heated. e) The silica gels were treated with $TiCl_4$, then heated.

TABLE 3. Separations of Diastereomers

Z	α ($k'_{erythro}/k'_{threo}$) Hypersil	Kromasil	Develosil not wash	Develosil acid wash	Sil(Al)	Sil(Ti)
NO_2[a]	1.13	1.13	NS	1.06	1.10	1.07
$PhSO_2$[a]	0.56	0.53	0.55	0.64	0.61	0.63
$PhSO_2$[f]	0.55	0.59	0.55	0.64	0.55	0.55
PhSO[b]	0.69	0.71	0.69	0.64	N.E.[h]	N.E.[h]
PhSO	1.91[d]	1.99[d]	1.77[d]	1.96[d]	1.98[g]	2.14[g]
PhNHOC	1.06[a]	1.70[a]	0.97[a]	1.19[a]	N.E.[h]	0.96[c]
PhNHOC[f]	3.43	3.77	3.77	3.47	2.75	2.41
TolNHOC[b]	1.59	1.75	1.47	1.23	1.35	1.40
TolNHOC	4.01[d]	4.45[d]	2.92[d]	4.36[d]	2.17[g]	2.45[g]

Mobile phase : Hexane : Ethyl acetate = a) 90:10, b) 80:20, c) 65:35.
Hexane : iso-propanol = d) 98: 2, f) 95: 5, g) 90:10 (v/v)
h) Not eluted in 60 minutes.

The diastereomers can be well resolved on all silica gels except those with nitro group. In the diastereomers including phenylsulfonyl group, erythro isomer was eluted faster than threo isomer, while in another diastereomers including amide and nitro functional group, threo isomer tended to be eluted faster than erythro isomer. Interestingly, in the diastereomer including the most hydrophilic phenylsulfinyl group, the elution orders were reversed in two different mobile phases and in a mobile phase including iso-propanol, threo isomer was eluted faster. A similar finding was also found out in the diastereomers including an amide group and in a mobile phase including iso-

propanol, threo isomer was eluted much faster than in the mixture of hexane and ethyl acetate. However, the overall retention behavior was not so complicated as expected.

As described earlier, those diastereomers tend to possess relatively fixed gauche conformation as shown in Figure 4, that was also confirmed by NMR measurement in a mixed solvent like a mobile phase. According to Newman projections, the erythro isomer seems to be more compact than dose the threo isomer because the erythro isomer is sterically more crowded in the molecule than the threo isomer. In other word, substituent groups including alkyl side chains are parted into two opposite directions in the threo isomer to afford relatively bulky conformation. Since the silica gels interact with planer and compact molecules more tightly, the compact erythro isomer may be retained longer on solid support when the substituent group which specially interact with adsorbent is not included. Since nitro group is not so hydrophilic and not so bulky substituent, the diastereomers including nitro group are not resolved well. In normal phase mode, alkyl substituent doesn't make big difference on the retention time, however p-methyl substituent on phenyl ring afford much better resolution in the case of the diastereomers including amide substituent. Those may also suggest a bulkiness of the diastereomer plays an important role for the molecular recognition. A typical chromatogram is demonstrated in Figure 5.

Although the diastereomer including phenylsulfonyl group gave fully opposite results, for the seemingly discrepancy results, the opposite elution order found out in the diastereomer including phenylsulfinyl group in the two different mobile phases seems to give one possible answer. As demonstrated in Table 1, phenylsulfinyl group showed relatively shorter retention time in alcoholic mobile phase than that in a mobile phase including ethyl acetate because of some solvation of mobile phase molecule to sulfoxide group. In a mobile phase including iso-propanol, the threo 2-phenylsulfinyl-3-undecanol was eluted faster than erythro form. When the adsorbent interact with a substituent group strongly, the bulkiness around the substituent group may prevent preferable interaction between the adsorbent and the stereoisomer and in this case the

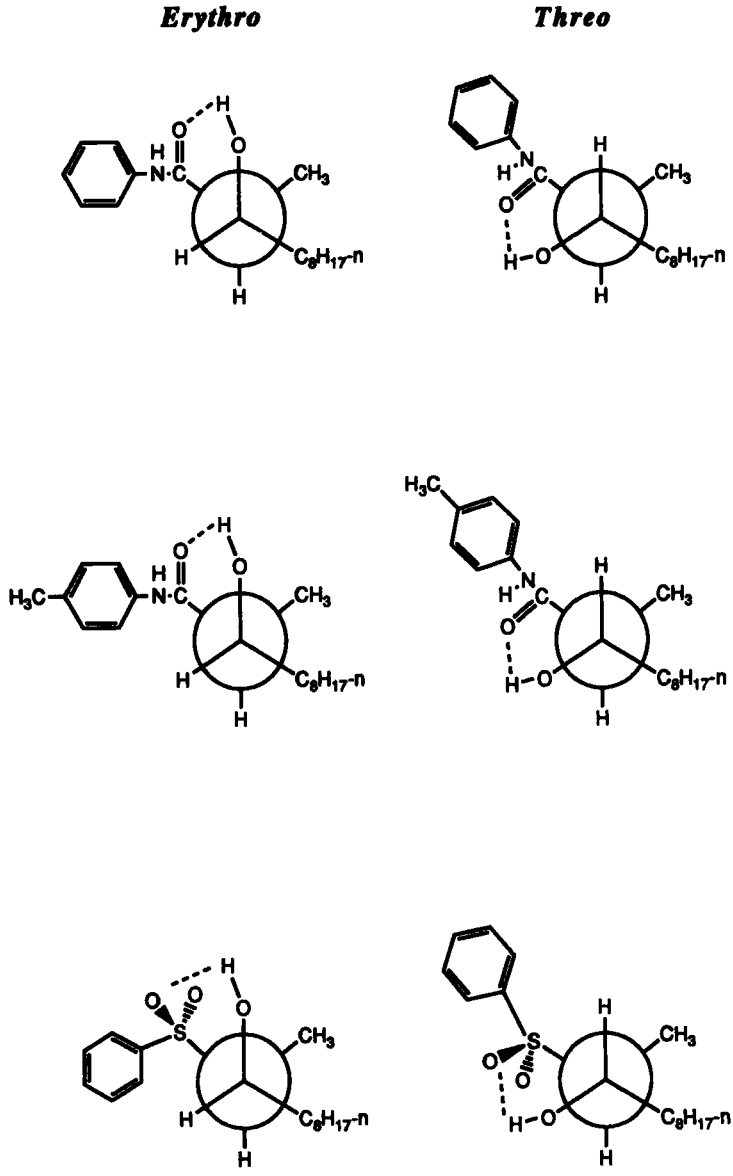


FIGURE 4. *Gauche* conformation of some alcohols

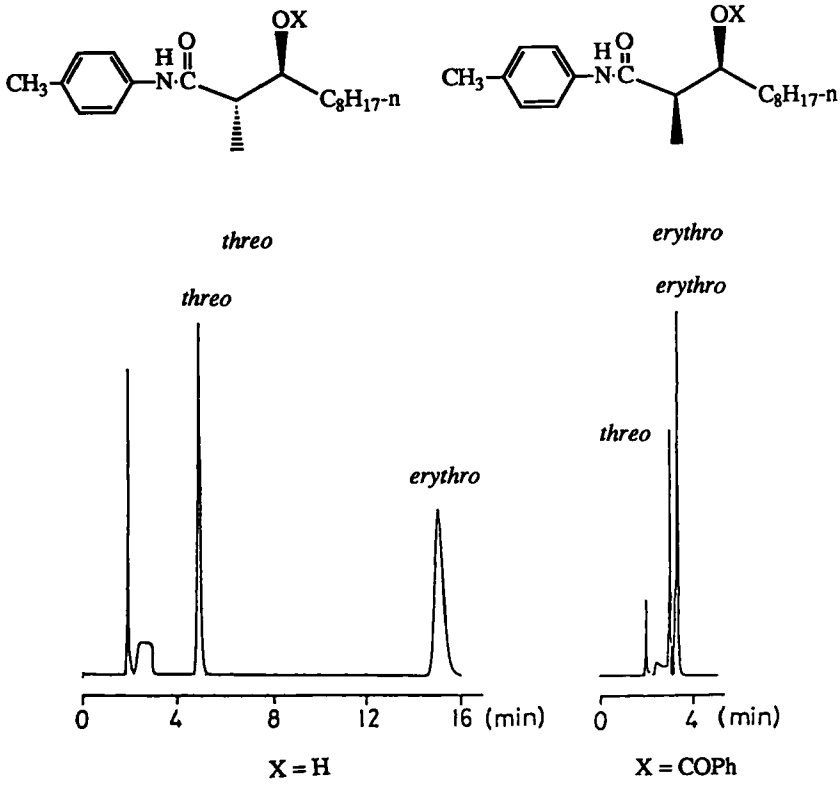


FIGURE 5. Separations of a nonprotected alcohol and a protected alcohol on silica gels.
chromatographic conditions
stationary phase; Develosil (acid washed)
mobile phase; hexane:iso-propanol=98:2
flow rate; 1 ml/min.
detection; UV 254 nm

threo isomer in which the substituent groups are parted from the bulky alkyl substituents may be retained longer than the erythro isomer. In addition, on the adsorbents including metal impurities which may lead a special interaction such as a chelation, opposite elution order and/or preferable retention of threo isomer are also found in the diastereomers including amide substituent. For the chelation, threo isomer is more

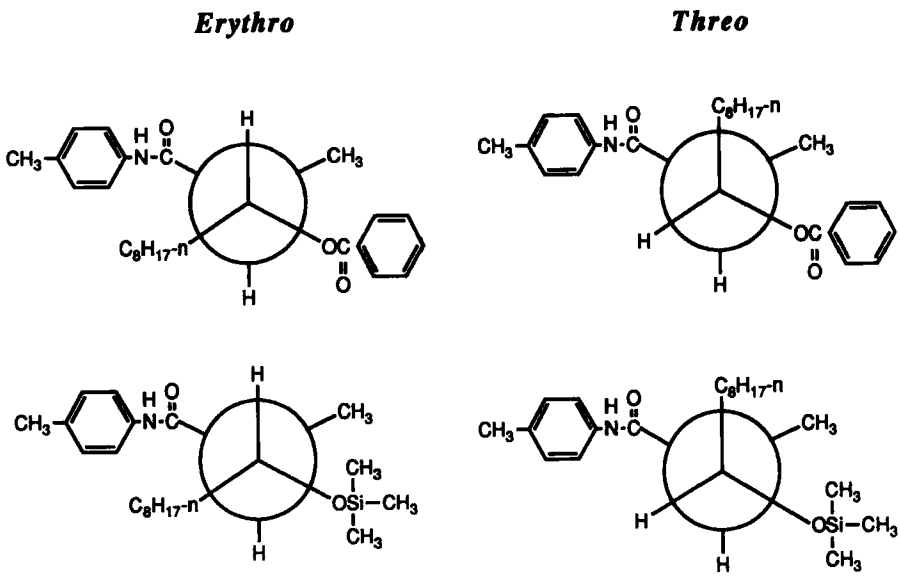


FIGURE 6. Conformation of some protected alcohols

preferable, because the hydroxy group and the hydrophilic substituent group are parted from alkyl substituents to permit an easy access to the adsorbent. A strong interaction on the metal included adsorbent was confirmed by no elution of some isomer within 60 minutes.

In the above diastereomers included intramolecular hydrogen bonding which tended to fix their conformation. In the next step, that intramolecular hydrogen bonding was destroyed through a chemical protection of the hydroxy group. For this porous, acetyl, benzoyl and trimethylsilly groups were employed. Acetyl and benzoyl group may interact with the adsorbent silica gels somehow, on the other hand, trimethylsilyl group is a just bulky group which prevent intramolecular hydrogen bonding. In this case, the diastereomer including an amide group was treated with the protective groups using an typical reaction condition (Figure 6).

The results are summarized in Table 4.

TABLE 4. Separation of Protected Diastereomer

X-	α ($k'_{erythro}/k'_{threo}$)					
	Hypersil	Kromasil	Develosil no wash	Develosil acid wash	Sil(Al)	Sil(Ti)
H-	4.01	4.45	2.92	4.36	2.17[a)]	2.45[a)]
CH_3OC-	2.33	2.44	2.11	2.19	2.40	2.53
PhOC-	1.47	1.51	1.74	1.52	1.71	1.75
$(CH_3)_3Si$-	NS	NS	NS	NS	NS	NS

Mobile phase ; Hexane : iso-propanol = 98 : 2 (v/v)
a) ; Hexane : iso-propanol = 80 : 20 (v/v)
NS; Not separated

As expected, the diastereomer protected with trimethylsilyl group can not be separated on any silica gels that may be due to poor interaction between hydrophobic silyl substituent instead of a nonprotected hydroxy group and the adsorbent. Of course, a protective group sometimes can help a resolution of the diastereomers, but in this case, the conformational change by the protection may be a dominant factor for bad resolution. Interestingly, elution order of the acetyl or benzoyl protected diastereomers were the same as nonprotected diastereomer, however, resolution was much worse for the protected diastereomer (Figure 5). Since the protected hydroxy group is sterically bulky and no longer possess an ability for intramolecular hydrogen bonding, it is not surprising the both diastereomer can not occupy the gauche conformation to tend to result in the anti conformation as shown in Figure 6. Although acetyl group and benzoyl group can also interact with the adsorbent because of a hydrogen acceptor property, in this case, threo isomer becomes more compact to be retained longer. However overall bulkiness of the both isomers doesn't seem to be so different and this may be a reason why protection of the hydroxy group make their isomer resolution worse as demonstrated in Figure 5. Moreover, no differences in the elution order and the resolution were found on both pure and the metal included silica gels, while nonprotected diastereomer were worse resolved on the metal included silica gels. That is normal because no chelation works on the protected diastereomers.

CONCLUSION

Using a simple technique, the separation of a really very important key compound for the syntheses of naturally occurring compounds can be done. Even if a diastereomer is acyclic, the elution order can be expected based on a few parameters in a normal phase mode with silica gels. Of course, there are some exceptions in the elusion order of diastereomers, however, it may be certified that HPLC technique is becoming one of the most effective techniques for both determinations of chemical and enantiomerical purity of compounds and a prediction of configuration of stereoisomers when the stereoisomers posses a certain conformation which is mainly determined by NMR technique. As described earlier, stereochemical purity of organic compounds as well as chemical purity shall become much more important for drug syntheses, because lots of drugs are chiral compounds and racemic mixture has been utilized for the most of drug in spite of a different virtue between racemates. Some chiral column for the preparative separation of racemic compounds become much cheaper than that 10 years ago, however, a contamination of cleft stationary phase into the solutes is a nonavoidable problems even if the nice resolution can be obtained. Therefore cheap and simple separation technique is still important for such organic syntheses because a certain diastereomeric intermediate must be in existence through the synthetic pathway. From this point of view, the primary separation work has been started. Many data should be obtained for the predictions of configuration of stereoisomers by chromatographic resolution without any exceptions, however, this work may be useful in development further the theory and/or rule of selectivity in separation of stereoisomers by a simple technique and another separations have been currently in progress.

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